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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: **METHYL BROMIDE. ID NO. 053201.** Evaluation of Micronucleus Assay in Rodent (In Vivo Inhalation Exposure).

Tox. Chem. No.	555
PC Code No.	053201
DP Barcode No.	D208053, D220502
Submission No.	S474691, S496109

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Linnea J. Hansen 11/8/95

To: Larry Schnaubelt, Manager, PM Team 72
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Through: John Doherty, Ph.D., D.A.B.T., Acting Section Head
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John Doherty 11/14/95

KB 11/27/95

I. CONCLUSIONS

An increased frequency of micronucleus formation in bone marrow or peripheral blood erythrocytes was observed in both rats and mice following inhalation exposure to methyl bromide. The results of the study are summarized below:

EXECUTIVE SUMMARY: In a rodent micronucleus induction study (MRID 43786501), 10/sex/dose BDF1 mice and F344 rats were exposed in vivo by inhalation to methyl bromide vapor at concentrations of 0, 154, 200, 260, 338 or 440 ppm (equivalent to 0, 0.597, 0.776, 1.008, 1.311 or 1.706 mg/L) for 6 hrs/day, 5 days/week for 14 days, or 10 exposures. Micronucleus (MN) induction was evaluated in bone marrow of rats and mice and in peripheral blood of mice.

In mice, significantly increased incidence of MN in bone marrow polychromatic erythrocytes (PCEs) was observed in males at 154 and 200 ppm (2.6- and 10.5-fold, respectively) and in females at 154 ppm (5.8-fold); smaller increases in MN frequency were observed in normochromatic erythrocytes (NCEs). Peripheral blood showed significant increases at 200 ppm in males (32.6-fold) and 154 ppm in females (2.6-fold); MN in NCE showed small increases. Mice exposed to ≥260 ppm were not assayed due to excessive mortality. In rats, MN in PCEs of bone marrow were increased at 338 ppm in males (13.6-fold; statistically significant) and in females at 260 and 338 ppm (3.3-fold; not statistically significant). Rats exposed to 440 ppm were not assayed due to excessive mortality.

This study is classified as Acceptable and fulfills the guideline requirement for mutagenicity testing (chromosomal aberrations; 84-2b) of methyl bromide. Several deficiencies related to reporting of the methods and results (see Discussion section of DER) did not preclude acceptance of the study or the conclusion that a positive effect on the micronucleus frequency was observed in two species.

Outstanding mutagenicity data: At this time, the only outstanding mutagenicity data requirement is a mouse heritable locus assay. This study requirement was triggered by a positive testicular DNA alkaline elution study in rats following inhalation exposure to methyl bromide (MRID 43180201; reviewed in HED doc. no. 011065). The micronucleus study reviewed in this document satisfies the guideline requirement for 84-2b, chromosomal aberrations.

II. ACTION REQUESTED

TB-1 received for evaluation a study on micronucleus frequency in rats and mice exposed in vivo by inhalation to methyl bromide vapor. The study was submitted by the Methyl Bromide Task Force to satisfy mutagenicity testing requirements under Guideline 84-2(b) for chromosomal abnormalities at the request of TB-I (see letter from L. Rossi to Kathryn Rosica, MBIP dated 3-16-94). **The study, from the Japan Bioassay Laboratory, Japan Industrial Safety and Health Association, was initially provided as a pre-publication draft (MRID 43383001) under the condition that the report remain confidential for one year or until publication. However, on 9/14/95 the published report (MRID 43786501) was received by the Agency (*Environ. Mut. Res. Commun.* 17:47-56 (1995).**

Reviewed by: Linnea J. Hansen, Ph.D. Linnea Hansen 11/8/95
Section IV, Tox. Branch I (7509C)
Secondary reviewer: Irving Mauer, Ph.D. [redacted] 11/13/95
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DATA EVALUATION REPORT

CHEMICAL: Methyl Bromide

PC No.: 053201 Tox. Chem. No.: 555

STUDY TYPE: Study on induction of micronuclei in rats and mice following in vivo inhalation exposure.

MRID NUMBER: 43786501 (pre-publication draft 43383001)

SYNONYM/CAS NO.: Bromomethane/74-83-9

SPONSOR: Chemical Manufacturers Association/Methyl Bromide Industry Panel, Washington, DC

TESTING FACILITIES: Japan Bioassay Laboratory, Japan Industrial Safety and Health Association, Hirasawa, Hadano,. Kanagawa, Japan

TITLE OF REPORT: Methyl Bromide - Micronuclei Induction of Methyl Bromide in Rats and Mice by Subchronic Inhalation Toxicity Test

AUTHORS: A. Araki, F. Kato, T. Matsushima, N. Ikawa and K. Nozaki

STUDY NUMBER: None assigned

REPORT ISSUED: Date not assigned (the study was apparently completed in 1986)

PUBLICATION CITATION: Environ. Mut. Commun. 17:47-56 (1995)

EXECUTIVE SUMMARY: In a rodent micronucleus induction study (MRID 43786501), 10/sex/dose BDF1 mice and F344 rats were exposed in vivo by inhalation to methyl bromide vapor at concentrations of 0, 154, 200, 260, 338 or 440 ppm (equivalent to 0, 0.597, 0.776, 1.008, 1.311 or 1.706 mg/L) for 6 hrs/day, 5 days/week for 14 days, or 10 exposures. Micronucleus (MN) induction was evaluated in bone marrow of rats and mice and in peripheral blood of mice.

In mice, significantly increased incidence of MN in bone marrow PCEs was observed in males at 154 and 200 ppm (2.6- and 10.5-fold, respectively) and in females at 154 ppm (5.8-fold); smaller increases in MN frequency were observed in NCE.

Peripheral blood showed significant increases at 200 ppm in males (32.6-fold) and 154 ppm in females (2.6-fold); MN in NCE showed small increases. Mice exposed to ≥260 ppm were not assayed due to excessive mortality. In rats, MN in PCEs of bone marrow were increased at 338 ppm in males (13.6-fold; statistically significant) and in females at 260 and 338 ppm (3.3-fold; not statistically significant). Rats exposed to 440 ppm were not assayed due to excessive mortality.

This study is classified as Acceptable and fulfills the guideline requirement for mutagenicity testing (chromosomal aberrations; 84-2) of methyl bromide. The study is acceptable despite numerous deficiencies in reporting of the methods and results (see Discussion section of DER) because a positive result was obtained in two species.

1. MATERIALS AND TEST ANIMALS

Methyl bromide in cylinders (99% pure; lot no. not indicated in report) was obtained from Taiho Sangyou Co. (Yokahama, Japan).

Male and female BDF1 mice and F344 rats were obtained from Charles River Japan (Atugi, Japan) and were acclimated for 2 weeks including 1 week of quarantine prior to initiation of exposures. Animals were 8 weeks of age at the start of the exposure period (body weights were not provided). Animals were housed individually in hanging wire-mesh cages in the exposure chamber and were given pellet diet (CRF-1, Oriental Yeast Co., Japan) and water ad libitum except during the exposure periods. No further details regarding animal care were included in the study report. The method of assignment of animals to each test group was not described.

2. TEST PERFORMANCE

A. Inhalation exposures: Whole body exposures were conducted in 1.06 m^3 exposure chambers designed by the Japan Bioassay Laboratory. Test material was metered into filtered air to give appropriate concentrations in the test chambers. Chamber air was analyzed using a gas chromatographic analyzer; sampling times were not indicated. The study report did not provide any information on particle size analyses (mass median aerodynamic diameter or MMAD and total mass concentration or TMC), chamber concentration analyses and conditions (temperature, humidity, oxygen levels, air changes), the T99 or how long animals were left in the exposure chambers following termination of exposure to allow clearance of test material.

A total of 10 rats and 10 mice per sex and exposure group were exposed by inhalation to methyl bromide vapor at chamber concentrations of 0, 154, 200, 260, 338 or 440 ppm

for 6 hrs, 5 days/week over a period of 2 weeks (total of 10 exposures). The study report did not indicate whether animals were observed for clinical signs or weighed during the study.

B. Micronucleus assays: Animals were sacrificed by an unspecified method. Femurs were removed from rats and mice and bone marrow cells were collected and smears prepared using the method of Schmid (1975). Blood was collected from the descending aorta of mice and smears were prepared on slides. The study report did not indicate when animals were sacrificed following the last exposure or provide experimental details, but stated that the methods of Schmid and of MacGregor et al. (1982, 1983) were used for performing these assays.

Slides of bone marrow erythrocytes for each animal were stained with May-Gruenwald Giemsa (mice) or methyl green pyronin (rats). Mouse peripheral blood cells were stained with Wright solution.

For bone marrow, at least 1000 polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) were scored per animal. For peripheral blood in mice, at least 1000 PCEs and 500 NCEs were scored. Data was analyzed statistically according to the method of Kastenbaum and Bowman. The criteria used to evaluate the data for positive response were not outlined in the study report.

Compliance statements: Signed and dated Quality Assurance and GLP statements were not included in the study report because the study was not conducted under GLP guidelines.

3. **RESULTS**

Chamber concentrations: The study report stated that animals were exposed to 0, 154, 200, 260, 338 or 440 ppm methyl bromide vapor. No other information on exposure conditions was provided.

Mortality: Mice exposed to 154 ppm methyl bromide survived except for one male. At 200 ppm, 5 males and 2 females survived. Only 1 male survived at 260 ppm; females and all animals exposed at higher levels died during the study. The study report indicated only survival after 14 days and did not provide information about the days of death.

All rats exposed to 260 ppm and lower survived. At 338 ppm, 3 males and 9 females survived. No animals survived exposure to 440 ppm.

Micronucleus assays: The results of the assays are shown below in Table 2 to 4 from the study report (attached). TB-I agreed with the study authors that exposure to methyl bromide increased the frequency of micronuclei in both mice and rats.

In mice, statistically significant increases in the incidence of micronuclei in PCEs were observed in bone marrow at 154 and 200 ppm in males (2.6- and 10.5-fold, respectively) and at 154 ppm in females (5.8-fold; a similar increase was observed at 200 ppm but only 1 animal was evaluated). MN incidence was also increased in NCEs (significantly in males at 154 and 200 ppm). There was no effect on PCE:NCE ratio in bone marrow. In peripheral blood of mice, significant increases in MN incidence in PCEs were observed at 200 ppm (males, 32.5-fold) and 154 ppm (females, 2.5-fold) but not at 200 ppm in females, where only 2 animals were examined. Small increases (significant in males at 200 ppm) were observed in NCE. Unlike the bone marrow, an increased PCE:NCE ratio was observed in exposed mice, suggesting toxicity of methyl bromide to circulating NCEs.

In rats exposed to 338 ppm methyl bromide, a statistically significant (13.7-fold) increase in MN/PCE in bone marrow was observed in males; in females, a 3.3-fold increase (not significant) was observed. There was no effect on PCE/NCE ratio.

4. DISCUSSION

The results of this study indicate that methyl bromide has the potential to cause chromosomal aberrations following short-term inhalation exposure as detected by micronucleus formation in bone marrow of rats and mice and in peripheral blood erythrocytes of mice. This effect was observed at exposure levels of 154 ppm in male and female mice and 260 ppm in female rats (and was more pronounced in males than females in both species).

Study deficiencies: There were significant deficiencies in the reporting of this study. Many of the details of study conduct and results were not included in the study report, such as chamber concentration analyses, animal housing, individual animal data, lot no. of test material, procedures and sampling times. However, because the results were positive for genotoxic potential of methyl bromide, TB-I considers the study to be acceptable for regulatory purposes.

Table 4
Bone marrow micronucleus test of methyl bromide in rats

Sex	Dose (ppm)	No. of rats	Scored MN/PCE	(‰) MNPCE	a) Ratio of PCE/NCE
Male	0	5	1/5384	0.19	1.01
	154	4	0/4206	<0.24	0.97
	200	4	1/4244	0.24	0.99
	260	5	2/5099	0.39	0.94
	338	3	8/3058*	2.60	0.96
Female	0	4	1/4362	0.23	0.71
	154	5	2/5165	0.39	0.75
	200	5	1/5251	0.19	0.71
	260	5	4/5271	0.76	0.78
	338	5	4/5296	0.76	1.00

Remarks a) May-Grunward Giemsa staining was used

Table is from study report, MRID 433830-01

Table 2
Bone marrow micronucleus test of methyl bromide in mice

Sex	Dose (ppm)	No. of mice	Scored MN/PCE	Scored MN/NCE	(‰) MNPCE	(‰) MNNCE	Ratio of PCE/NCE
Male	0	5	12/5032	19/5419	2.4	3.5	0.93
	154	5	37/5992*	51/5398*	6.2	9.5	1.11
	200	5	149/5927*	97/5596*	25.1	17.3	1.06
Female	0	4	4/4073	6/4205	1.0	1.4	0.94
	154	5	30/5180*	21/5343	5.8	3.9	0.96
	200	1	7/1712	4/1005	5.0	4.0	1.71

Table 3
Peripheral blood micronucleus test of methyl bromide in mice

Sex	Dose (ppm)	No. of mice	Scored MN/PCE	Scored MN/NCE	(‰) MNPCE	(‰) MNNCE	Ratio of PCE/NCE
Male	0	6	2/3000	5/6500	0.7	0.8	0.007
	154	7	10/3581	17/7863	2.8	2.2	0.072
	200	4	46/2018*	19/4452*	22.8	4.3	0.100
Female	0	8	16/4040	11/8594	4.0	1.3	0.012
	154	8	43/4144*	10/8454	10.4	1.2	0.136
	200	2	7/1043	9/2222	6.7	4.1	0.109

Tables are from study report, MRID 433830-01

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HED File Code	**13000 Tox Reviews**
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Accession Number:	**412-01-0073**

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